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Trends in the prevalence of COVID-19 infection in Rio Grande do Sul, Brazil: repeated serological surveys

Pedro Curi Hallal (https://orcid.org/0000-0003-1470-6461) [1]
Bernardo L Horta (https://orcid.org/0000-0001-9843-412X) [1]
Aluísio J D Barros (https://orcid.org/0000-0002-2022-8729) [1]
Odir A Dellagostin (https://orcid.org/0000-0003-2803-4088) [1]
Fernando P Hartwig (https://orcid.org/0000-0003-3729-0710) [1]
Lúcia C Pellanda (https://orcid.org/0000-0002-8776-2248) [2]
Cláudio José Struchiner (https://orcid.org/0000-0003-2114-847X) [3]
Marcelo N Burattini (https://orcid.org/0000-0002-5407-6890) [4]
Mariângela Freitas da Silveira (https://orcid.org/0000-0002-2861-7139) [1]
Ana M B Menezes (https://orcid.org/0000-0002-2996-9427) [1]
Fernando C Barros (https://orcid.org/0000-0001-5973-1746) [1]
Cesar Gomes Victora (https://orcid.org/0000-0002-2465-2180) [1]

Abstract *COVID-19, the disease produced by the virus SARS-CoV-2, has spread quickly throughout the world, leading the World Health Organization to first classify it as an international health emergency and, subsequently, declaring it pandemic. The number of confirmed cases, as April 11, surpassed 1,700,000, but this figure does not reflect the prevalence of COVID-19 in the population as, in many countries, tests are almost exclusively performed in people with symptoms, particularly severe cases. To properly assess the magnitude of the problem and to contribute to the design of evidence-based policies for fighting COVID-19, one must accurately estimate the population prevalence of infection. Our study is aimed at estimating the prevalence of infected individuals in the state of Rio Grande do Sul, Brazil, to document how fast the infection spreads, and to estimate the proportion of infected persons who present or presented symptoms, as well as the proportion of asymptomatic infections. Four repeated serological surveys will be conducted in probability samples of nine sentinel cities every two weeks. Tests will be performed in 4,500 participants in each survey, totaling18,000 interviews. Interviews and tests will be conducted at the participants' household. A rapid test for the detection of antibodies will be used; the test was validated prior to the beginning of the fieldwork.*
Key words *COVID-19, Infection, Prevalence, Population-based study, Brazil*

[1] Programa de Pós-Graduação em Epidemiologia, Universidade Federal de Pelotas. R. Marechal Deodoro 1160, Centro. 96020-220 Pelotas RS Brasil. prchallal@gmail.com
[2] Fundação Universidade Federal de Ciências de Saúde de Porto Alegre. Porto Alegre RS Brasil.
[3] Universidade do Estado do Rio de Janeiro. Rio de Janeiro RJ Brasil.
[4] Universidade de São Paulo. São Paulo SP Brasil.

Introduction

SARS-CoV-2 belongs to a family of viruses that may cause diseases in humans and animals. Since the detection of the first case in China, in the end of 2019, the virus has spread quickly throughout the world. On January 30, the World Health Organization (WHO) classified the disease produced by the virus, COVID-19, as an international health emergency. On March 11, WHO declared that there was a pandemic of COVID-19, with approximately 118,000 cases in 114 countries and territories. On April 11, the number of confirmed cases surpassed 1,700,000 in practically all countries and territories, with a confirmation of more than 103,000 deaths[1,2].

It is necessary, however, to consider that available official statistics on the evolution of the virus suffer from a number of limitations, particularly the absence of information on the prevalence of infection at the population level. For example, in the WHO situational report of April 10, 2020[3], there was the confirmation of 143,626 people with a COVID-19 positive test in Italy, a country with a population of 60.5 million inhabitants. Dividing the official number of infected cases by the size of the population, the prevalence of infections by SARS-CoV-2 would be 0.24%. However, testing for SARS-CoV-2 is not performed in a random way in the Italian population, where people with symptoms are much more likely to be tested than those without symptoms In the small village of Vo, in Northern Italy, all 3,300 inhabitants were tested, and in 3% the test resulted positive, most of them without symptoms[4].

In Iceland, where testing the population regardless of the occurrence of symptoms was stimulated, 3,787 persons had been tested until March 18, with 218 (5.8%) presenting positive results[5]. Even this estimation should be considered with caution, due to the well-known phenomenon of diagnostic bias[6], in which persons with symptoms may have opted to be tested with higher frequency than those without symptoms. When the analysis was performed specifically in the 1,800 tests conducted among asymptomatic volunteers, only 19 (1.1%) tested positive[5]. The prevalence also depends on the stage of the epidemics and it tends to increase with time. A recent survey showed a prevalence of infection of 14% in the German city of Gangelt, considered a disease focus after Carnival festivities[6], while in South Korea, more than half million people were tested in health services and 2.1% were positive[7].

In Epidemiology, to estimate the magnitude of a health problem in the whole population, and not in specific subgroups of people, suspected of being sick[8,9], is the first step to develop evidence-based effective public health strategies. To estimate the proportion of infected persons in the whole population is especially relevant with COVID-19, as it seems that more than 60% of the persons infected by the SARS-CoV-2 present light symptoms or are even asymptomatic[10], even though they may transmit the disease. Moreover, in the current situation of restrictive policies regarding social contact, to have precise information on the prevalence of the infection in the population and, consequently, the number of persons susceptible to get infected, will be essential to make plans for the gradual return to normal activities.

Objectives

The aims of the project are: (1) To estimate the proportion of the population of Rio Grande do Sul State that present antibodies for SARS-CoV-2; (2) to determine the proportion of the individuals positive for antibody test that presented subclinical or asymptomatic infections; (3) to evaluate the most common symptoms reported by the positive subjects; (4) to analyze the evolution of the prevalence of the infection through four consecutive surveys; (5) to allow precise estimations of case-fatality rates, by using the correct proportion of infected people; (6) to define the necessary number of low, medium, and high complexity hospital resources needed to confront the pandemic, through mathematical models obtained using precise prevalence estimates; (7) to allow the subsequent design of strategies to ease the measures of social distancing, based on the obtained estimations of infections in the community.

Population and sampling

Population-based repeated serological surveys will be carried out in nine sentinel cities, with a design recommended by WHO[11]. The selection of sentinel municipalities is justified by the little time available and the limited availability of diagnostic tests. Eight municipalities, the most populous cities in each intermediate subregions defined by the Brazilian Institute of Geography and Statistics (IBGE) of Rio Grande do Sul, will be included. The ninth study area is the biggest municipality of the metropolitan area, besides Porto Alegre itself, the State's capital. The eight municipalities of the intermediate subregions are: Porto Alegre, Pelotas, Santa Ma-

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ria, Uruguaiana, Ijuí, Passo Fundo, Caxias do Sul e Santa Cruz do Sul (Figure 1). Given the importance of the metropolitan area of Porto Alegre, the municipality of Canoas will be also included in the sample, as it is the third most populous of the State, after Porto Alegre and Caxias do Sul. In each survey, 500 interviews will be conducted in each stratum, totaling 4,500 interviews by survey and 18,000 interviews (and testing) in the whole study.

The four surveys will be carried out according to the timetable shown in Figure 2. The duration of data collection in each survey will be 2-3 days. The sampling scheme consists of a multiple stage probabilistic sample: in each sentinel municipality 50 census tracts will be selected with probability proportional to size, respecting the order of tracts defined by IBGE, that starts in central areas and radiate to the periphery of the city and city districts. Maps of census tracts updated in 2019 by IBGE, including all addresses for each sector, will be used to conduct a random sampling selection of 10 households in each tract. In case of refusal by the inhabitants of the selected household, other household will be added to the sample.

In each sampled household, all inhabitants will be counted by the interviewer, and their age and sex will be collected, and after that, one of them will be randomly chosen. If this person is not at home in the moment of the visit, the interviewer will return to that household at the end of the day for a second attempt. If the person is still absent, another resident of the household will be randomly selected. The same procedure will take place in case of refusal by the chosen household residents. All information about absences, and refusals, by the chosen resident or the household, will be registered to allow the estimation of non-response rates. In every new survey, the sampling will use the same census tracts, but different households.

Table 1 shows, considering a sample of 4,500 persons in the nine municipalities, the precision of the estimates for different levels of prevalence of infection for the State as a whole, and for each municipality.

Testing and questionnaires

The detection of COVID-19 will be made using the WONDFO SARS-CoV-2 Antibody Test.

Figure 1. Map of Rio Grande do Sul State, with municipalities included in the population-based serological study on the prevalence of COVID-19.

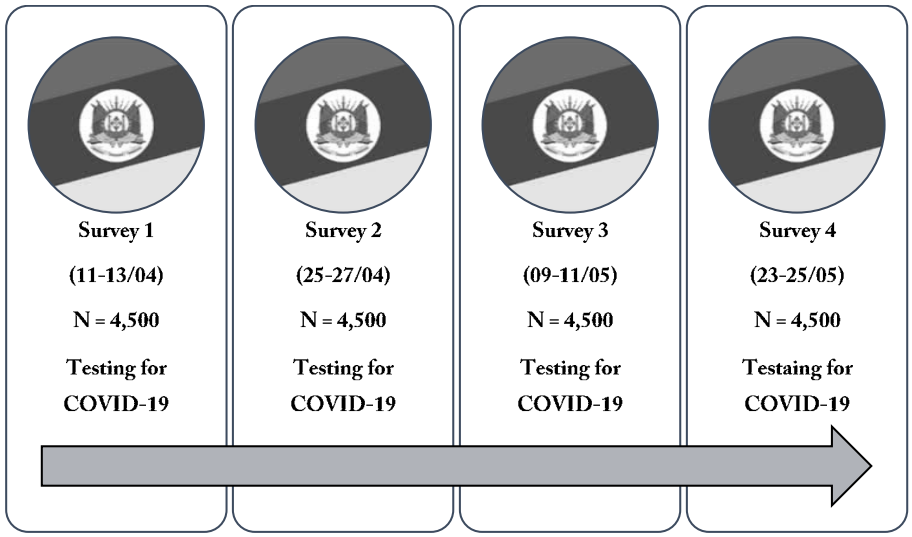

Figure 2. Timetable for data collection. Population-based serological study of the prevalence of COVID-19 in Rio Grande do Sul.

Table 1. Parameters and estimations used for calculating sample size. Population-based serological study of the prevalence of COVID-19 in Rio Grande do Sul.

Survey	% of infection	N	Total precision (pp)	Precision in each stratum (pp)
1	3%	4.500	0,50	1,50
2	5%	4.500	0,60	1,70
3	10%	4.500	1,00	2,50
4	20%	4.500	1,20	3,50

pp: percentage points.

This is based on the principle of immune essay of lateral flow to detect IgG/IgM antibodies against SARS-CoV-2 in human total blood, serum and plasma. The validation study provided by the producer included 596 participants and identified a sensitivity of 86.4% and specificity of 99.6%[12]. Our research team is conducting a validation study with the Rio Grande do Sul population, with individuals who had already been submitted to the PCR test. Although data collection is still on its way, preliminary results indicate a sensitivity over 70% and specificity over 95%. The test identifies the presence of immunoglobulins against SARS-CoV-2, without a discrimination if they are IgM or IgG. These antibodies might not be detectable in the first days after contagion; therefore, the test has scarce diagnostic value for acute cases and may present false negative results in early phases of the infection.

The interview team will be trained to conduct the antibody test with a blood sample obtained by finger prick. Besides the COVID-19 testing, the following information will be collected from each participant: sex, age, number of years of education of the sampled person, number of years of education of the person with the highest educations level in the household, self-referred skin color, and symptoms potentially related to COVID-19 (cough, fever, sore pain, breathing difficulties, palpitations, change in tasting and smelling, vomiting, diarrhea), all of them in the 15 days prior to the interview. Also, the following medical conditions related to prognosis of COVID-19 will be evaluated: high blood pres-

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sure, diabetes, asthma, cardiac diseases. Utilization of health care services in the same time period will also be inquired, as well as the level of adoption of the measures of social distancing (total, partial, no adoption).

Data analysis

Prevalence results will be stratified by sex, age, skin color, education level of the most educated person in the household, number of residents in the household, and number of people over 60 years of age in the household. Clinical symptoms presented by participants who tested positive and negative for the test will be analyzed, Also, we will estimate the proportion of asymptomatic infections. The results of the study will be compared with official data on notified cases, hospitalizations, and deaths due to COVID-19 in the nine municipalities, to estimate case-fatality rates and under notification of cases. The anonymized database will be made available for other researchers soon after data cleaning, consistency checks and initial analysis of the results.

Ethical aspects

All individuals sampled for the serological survey will be informed about the objectives of the study, possible risks and advantages. Blood samples and information will be collected only after the participant signed a free informed consent form, Individuals testing positive will be referred to the Secretary of Health of the municipality for necessary actions. All biological safety measures will be taken to protect the health of the field workers.

The study carries minimal health risks for the participants, as the only procedures will be data collection and blood test. If the participant feels any discomfort with the procedures, he/she will be free to leave the study at any time. The benefits of the study will be direct and indirect. In relation to the former, based on the test results the participants who tested positive will have the opportunity to receive proper management of their condition. Regarding the latter, the results of the study will provide more precise information on COVID-19, allowing the development of strategies to fight the pandemic with medical and preventative actions.

The project protocol was approved by the National Committee of Ethics in Research (CONEP).

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Collaborations

PC Hallal, BL Horta, AJD Barros, OA Dellagostin, FP Hartwig, LC Pellanda, CJ Struchiner, MN Burattini, MF Silveira, AMB Menezes, FC Barros and CG Victora were involved in all phases of the study. PC Hallal, BL Horta, AJD Barros, OA Dellagostin, FP Hartwig, LC Pellanda, CJ Struchiner, MN Burattini, MF Silveira, AMB Menezes, FC Barros and CG Victora were involved in all phases of the study.

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